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ORIGINAL PAPER

Differential Contribution of L-, N-, and P/Q-type Calcium Channels to $[Ca^{2+}]_i$ Changes Evoked by Kainate in Hippocampal Neurons

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Abstract We investigated the contribution of L-, N- and P/Q-type Ca^{2+} channels to the $[Ca^{2+}]_i$ changes, evoked by kainate, in the cell bodies of hippocampal neurons, using a pharmacological approach and Ca^{2+} imaging. Selective Ca^{2+} channel blockers, namely nitrendipine, ω-Conotoxin GVIA (ω-GVIA) and ω-Agatoxin IVA (ω-AgaIVA) were used. The $[Ca^{2+}]_i$ changes evoked by kainate presented a high variability, and were abolished by NBQX, a AMPA/kainate receptor antagonist, but the *N*-methyl-D-aspartate (NMDA) receptor antagonist, D-AP5, was without effect. Each Ca^{2+} channel blocker caused differential inhibitory effects on $[Ca^{2+}]_i$ responses evoked by kainate. We grouped the neurons for each blocker in three subpopulations: (1) neurons with responses below 60% of the control; (2) neurons with responses between 60% and 90% of the control, and (3) neurons with responses above 90% of the control. The inhibition caused by nitrendipine was higher than the inhibition caused by ω-GVIA or ω-AgaIVA. Thus, in the presence of nitrendipine, the percentage of cells with responses below 60% of the control was 41%, whereas in the case of ω-GVIA or ω-AgaIVA the values were 9 or 17%, respectively. The results indicate that hippocampal neurons differ in what concerns their L-, N- and P/Q- type Ca^{2+} channels activated by stimulation of the AMPA/kainate receptors.

Keywords Voltage-sensitive calcium channels · $[Ca^{2+}]_i$ changes · AMPA and kainate receptors · Hippocampal neurons

Introduction

Ionotropic glutamate receptors are ligand-gated ion channels, permeable to cations, and have been classified into three subtypes: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) and kainate receptors. NMDA receptors are responsible for the slow component of excitatory postsynaptic potentials, whereas AMPA and kainate receptors mediate fast excitatory transmission. These receptors are involved in important physiological and pathological processes, such as cell differentiation and growing, learning and memory, and excitotoxicity [1].

The activation of ionotropic glutamate receptors causes a substantial Na^+ entry into the cell, and the subsequent cell depolarisation triggers the opening of voltage-sensitive Ca^{2+} channels (VSCCs) and Ca^{2+} entry, which may also occur directly through NMDA receptors, as well as through Ca^{2+} permeable AMPA and kainate receptors, depending on their subunit composition.

In nerve cells, L-type Ca^{2+} channels are predominantly located in cell bodies and dendrites [2–4]. N-type Ca^{2+} channels are mainly located in nerve terminals and dendrites, although they can also be found in cell bodies [5–7]. P-type and Q-type Ca^{2+} channels are predominantly located in nerve terminals, but they may also be present in dendrites and cell bodies [2, 8–10]. The pharmacological

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distinction between P- and Q-type Ca^{2+} channels is difficult because they are both composed by the α_{1A} subunit, and therefore they are usually named P/Q-type Ca^{2+} channels [11, 12].

We have previously shown that VSCCs, mainly L- and N-type Ca^{2+} channels, have a predominant role in the $[Ca^{2+}]_i$ changes evoked by kainate in cultured hippocampal neurons measured in cell populations [13]. The $[Ca^{2+}]_i$ responses evoked by kainate have a high degree of variability among individual hippocampal neurons [14], which is likely due to a differential expression of AMPA and kainate receptors. These variations might have consequences in synaptic efficacy and information processing. However, the variability in $[Ca^{2+}]_i$ responses may be also due to a differential distribution of VSCCs among hippocampal neurons. In the present work we investigated and quantified the contribution of several Ca^{2+} channels to the Ca^{2+} transients induced by AMPA/kainate receptor activation in single hippocampal neurons, by Ca^{2+} imaging.

Experimental Procedure

Materials

Neurobasal medium, B27 supplement, gentamicin and trypsin (USP grade) were purchased from GIBCO BRL, Life Technologies, Scotland. DNase (DN-25) was purchased from Sigma Chemical, St. Louis, MO, USA. Kainate, 1,2,3,4-Tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide (NBQX) and D(−)-2-amino-5-phosphonopentanoic acid (D-AP5) were purchased from TOCRIS, Bristol, UK. The acetoxymethyl ester of Fura-2 (Fura-2/AM) and Pluronic F-127 were purchased from Molecular Probes, Leiden, The Netherlands. Nitrendipine was a kind gift of Dr G. Terstappen, from Bayer AG, Germany. ω-GVIA was obtained from Peninsula Laboratories, Belmont, CA, USA, and ω-AgaIVA was from Peptide Institute, Osaka, Japan. All other reagents were from Sigma Chemical, St. Louis, MO, USA or from Merck-Schuchardt, Germany.

Cell Culture

All procedures involving animals were conducted in accordance with the "Principles of laboratory animal care" (NIH publication No. 86-23, revised 1985) statement for the use of animals.

Hippocampal neurons were obtained from the hippocampi of E18-E19 Wistar rat embryos, after treatment with trypsin (1.0 mg/ml; 15 min; 37°C) and deoxyribonuclease I (0.15 mg/ml) in Ca^{2+}- and Mg^{2+}-free Hank's balanced salt solution (137 mM NaCl, 5.36 mM KCl, 0.44 mM KH_2PO_4, 0.34 mM $Na_2HPO_4 \cdot 2H_2O$, 4.16 mM $NaHCO_3$, 5 mM glucose, supplemented with 0.001% phenol red, 1 mM sodium pyruvate, 10 mM HEPES, pH 7.4). Hippocampal neurons were cultured in B27-supplemented Neurobasal medium (GIBCO), a serum-free medium combination [15], supplemented with glutamate (25 μM), glutamine (0.5 mM) and gentamicin (0.12 mg/ml). Cultures were kept at 37°C in a humidified incubator in 5% CO_2/95% air, for 7–8 days, the time required for maturation of hippocampal neurons.

The cells were plated at a density of 45×10^3 cells/cm^2 on poly-D-lysine-coated coverslips.

Fura-2 Fluorescence Imaging in Individual Cells

The changes in intracellular free Ca^{2+} concentration $[Ca^{2+}]_i$ of individual cells were measured using the fluorescent probe Fura-2 [16]. Hippocampal neurons were incubated with 5 μM Fura-2/AM (Molecular Probes) and 0.02% Pluronic F-127 (Molecular Probes) for 30 min at 37°C, in Krebs buffer (142 mM NaCl, 4 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM glucose, 10 mM Hepes-Na, pH 7.4) supplemented with 0.1% bovine serum albumine (BSA; Calbiochem-Boehringer). After incubation, the coverslips were rinsed with Krebs buffer and placed in a perfusion chamber on the stage of an inverted Nikon Diaphot fluorescence microscope.

The experiments were conducted under continuous perfusion with warm Krebs buffer (30°C) with or without drugs, as indicated in the figure legends. Each recording lasted 75 s, and neurons were stimulated 20 s after image acquisition. The fluorescence changes were recorded with a multiple excitation Magical imaging system (Applied Imaging, UK). Hippocampal neurons were alternately excited at 340 and 380 nm using a switching filter wheel, and the emitted fluorescence, collected with a 40× objective (Nikon) was driven to a SIT camera (Silicon Intensified Target; Photonics Science), after passing through a 510 nm bandpass filter. Image analysis was performed with the Magical imaging system. Briefly, the background fluorescence at each wavelength (340 and 380 nm) was subtracted and the ratio of the fluorescence images on a pixel-by-pixel basis was obtained. The data were stored as 8-bit pseudocolor images. Areas of the cell bodies were drawn and the averaged value of pixel intensities was evaluated at each time point, in order to obtain fluorescence ratio versus time plots for all areas defined. The results were expressed as the ratio of fluorescence intensity with excitation at 340 and 380 nm versus time.

Design of Ca^{2+} Experiments

Each experiment consists of two independent recordings, and the first recording was considered an internal control for each cell. The hippocampal neurons were stimulated

twice with kainate (t = 20 s), which was present until the end of each recording (t = 75 s). The cells were allowed to recover for 15 min between the first and second stimulus, and each drug tested was present 5 min before the second stimulus, until the end of the experiment.

Data Analysis

The data are expressed as mean ± SEM. Significance was considered at $P < 0.05$ using a two sample Student's t test.

Results

Characterization of the $[Ca^{2+}]_i$ Responses Evoked by Kainate in Hippocampal Neurons

We analyzed the $[Ca^{2+}]_i$ changes evoked by 100 μM kainate in the cell bodies. As expected, there was a high variability among cells in terms of $[Ca^{2+}]_i$ changes. We considered the $[Ca^{2+}]_i$ changes as the differences between the peak, after stimulus, and the basal value of the 340 nm/380 nm fluorescence ratio. In all experiments, the first stimulus was used as an internal control.

In control experiments, hippocampal neurons were stimulated twice with kainate, in the absence of calcium channel blockers. The second $[Ca^{2+}]_i$ response was similar to the first one (95.8 ± 1.5% of the control; Fig. 1a) in all hippocampal neurons analyzed ($n = 48$). The AMPA/kainate receptor antagonist, NBQX (10 μM), abolished the $[Ca^{2+}]_i$ changes (Fig. 1b). The presence of D-AP5 (100 μM), a NMDA receptor antagonist, did not cause any inhibitory effect on the $[Ca^{2+}]_i$ changes (97.0 ± 1.3% of the control; $n = 31$ neurons), thus excluding NMDA receptor involvement in the $[Ca^{2+}]_i$ response to kainate.

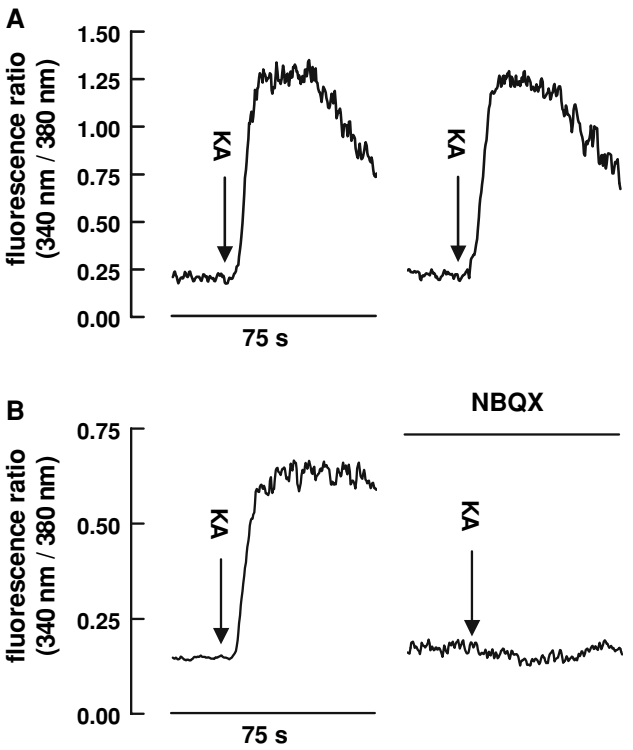

Fig. 1 Representative recordings of kainate-evoked $[Ca^{2+}]_i$ changes, measured with the fluorescent probe Fura-2, in single hippocampal neurons. (**A**) Control; (**B**) Effect of NBQX (antagonist of AMPA/kainate receptors). The results are expressed as the ratio of fluorescence intensity with excitation at 340 and 380 nm (emission at 510 nm) versus time. Each experiment consists of two independent recordings (75 s each), and the first recording was considered an internal control for each cell. The $[Ca^{2+}]_i$ changes were evoked by 100 μM kainate, and were analyzed in cell bodies. The cells were stimulated twice with kainate (t = 20 s), with a 15 min recovery interval between the first and the second stimulus. NBQX was present 5 min before the second stimulus, until the end of the experiment. (**A**) In control experiments, hippocampal neurons were stimulated twice with kainate, with no drug present during the second stimulus. The second recording was similar to the first one in all 48 cells analyzed from three independent cell cultures. (**B**) NBQX abolished the kainate evoked-$[Ca^{2+}]_i$ changes in the 38 cells analyzed from two independent cell cultures

Contribution of L-type Ca^{2+} Channels to the $[Ca^{2+}]_i$ Changes Evoked by Kainate

The contribution of L-type Ca^{2+} channels to the $[Ca^{2+}]_i$ changes evoked by kainate was analyzed by stimulating neurons in the presence of nitrendipine (10 μM). The L-type Ca^{2+} channel blocker, nitrendipine, inhibited the $[Ca^{2+}]_i$ changes to 60.5 ± 1.9% of the control ($n = 58$ cells; $P < 0.05$). Since the inhibitory effect of nitrendipine was very heterogeneous in the cell population, the cells were grouped in three subpopulations, as follows: responses to kainate in the presence of nitrendipine below 60% of the control (subpopulation 1), between 60% and 90% of the control (subpopulation 2), and above 90% of the control (subpopulation 3) (Table 1). Considering the first group

Table 1 Effect of nitrendipine on $[Ca^{2+}]_i$ changes evoked by kainate in the total population, and in subpopulations 1, 2 and 3

Δ $[Ca^{2+}]_i$ (% of control ± SEM)			
Total population	Subpopulation 1 (<60%)	Subpopulation 2 (60–90%)	Subpopulation 3 (>90%)
60.5 ± 1.9% (100%)	46.2 ± 1.9% (41.4%)	69.9 ± 1.1% (56.9%)	94% (1.7%)

The percentage of hippocampal neurons belonging to each population is indicated in parenthesis

of cells, responses below 60% of the control (first stimulus), the $[Ca^{2+}]_i$ response evoked by kainate, in the presence of nitrendipine, was 46.2 ± 1.9% of the control (Fig. 2a).

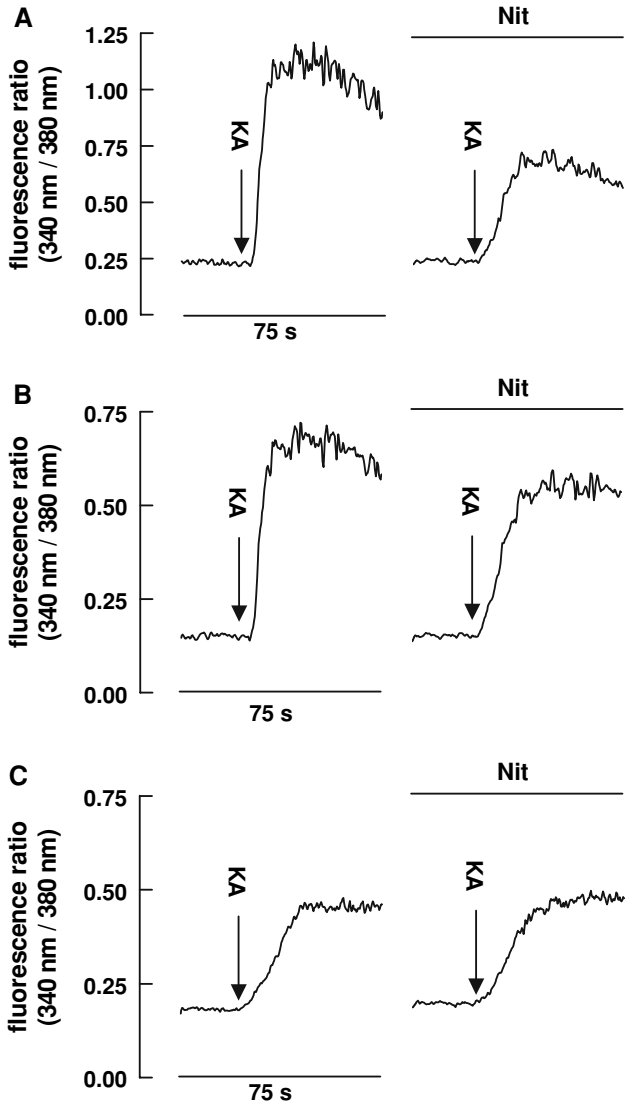

Fig. 2 Effect of nitrendipine (L-type Ca^{2+} channel blocker) on the $[Ca^{2+}]_i$ changes evoked by kainate (100 μM) in single hippocampal neurons. The results are expressed as the ratio of fluorescence intensity with excitation at 340 and 380 nm (emission at 510 nm) versus time. Each experiment consists of two independent recordings (75 s each), and the first recording was considered an internal control for each cell. The $[Ca^{2+}]_i$ changes were considered as the differences between the peak, after stimulus, and the basal value of the 340 nm/380 nm fluorescence ratio. The $[Ca^{2+}]_i$ changes were analyzed in cell bodies. Nitrendipine (Nit.; 10 μM) was present 5 min before the second stimulus, until the end of each experiment. Since the inhibitory effect of nitrendipine was very heterogeneous, the cells were grouped in three subpopulations. The recordings shown above are representative of each cell population, with $[Ca^{2+}]_i$ responses below 60% of the control (**A**), between 60% and 90% of the control (**B**), and above 90% of the control (**C**). A total of 58 cells, from four independent cell cultures, were analyzed, and the quantification of the data is shown in Table 1

This group of cells represents 41.4% of the cells stimulated in the presence of nitrendipine. In the second group of cells (responses between 60% and 90% of the control), which represents 56.9% of cells (33 neurons), nitrendipine inhibited the $[Ca^{2+}]_i$ increase to 69.9 ± 1.1% of the control (Fig. 2b). The $[Ca^{2+}]_i$ response was above 90% of the control (94%) in just one cell (Fig. 2c), thus indicating that L-type Ca^{2+} channels have a major contribution to the $[Ca^{2+}]_i$ changes upon non-NMDA glutamate receptors activation in the majority of hippocampal neurons.

Contribution of N- and P/Q-type Ca^{2+} Channels to the $[Ca^{2+}]_i$ Changes Evoked by Kainate

The contribution of N- and P/Q-type Ca^{2+} channels to the $[Ca^{2+}]_i$ changes evoked by kainate were also investigated, by stimulating the cells in the presence of ω-GVIA or ω-AgaIVA, respectively. The $[Ca^{2+}]_i$ changes were inhibited 20.1 ± 1.7% ($P < 0.05$) or 17.2 ± 2.2% ($P < 0.05$) when neurons were stimulated in the presence of ω-GVIA (500 nM) or ω-AgaIVA (100 nM), respectively. Again, due to the variability of the inhibitory effects caused by these Ca^{2+} channel blockers, the cells were grouped in three different populations, as indicated in Tables 2 and 3.

Relatively to the contribution of N-type Ca^{2+} channels to the $[Ca^{2+}]_i$ changes, 76 neurons were analyzed. In a small percentage of neurons (9.2%; seven cells), the $[Ca^{2+}]_i$ changes evoked by kainate, with ω-GVIA present, were below 60% of the control (48.1 ± 3.7% of the control; Fig. 3a). In the second population of neurons ($[Ca^{2+}]_i$ responses between 60% and 90% of the control), which represents the majority of neurons (73.7% of neurons), ω-GVIA inhibited the $[Ca^{2+}]_i$ increase to 79.6 ± 0.9% of the control (Fig. 3b; Table 2). In 13 neurons (17.1% of neurons) the responses were not significantly affected by the presence of ω-GVIA (98.4 ± 1.5% of the control; Fig. 3c).

The results obtained when neurons were stimulated in the presence of the P/Q-type Ca^{2+} channel blocker, ω-AgaIVA, were similar to those obtained with ω-GVIA. In these experiments, 48 neurons were analyzed. Considering the first population (neurons with responses below 60% of the control), the presence of ω-AgaIVA inhibited the $[Ca^{2+}]_i$ increase to 51.7 ± 2.9% of the control (Fig. 4a), corresponding to 16.7% of neurons (eight cells). In the second population, that includes the majority of neurons (66.7% of cells), the $[Ca^{2+}]_i$ increase was inhibited to 72.3 ± 1.5% of the control (Fig. 4b). In the third population (eight neurons; 16.6% of cells), ω-AgaIVA was without effect, and the $[Ca^{2+}]_i$ response was 95.8 ± 1.8% of the control (Fig. 4c; Table 3).

Table 2 Effect of ω-GVIA on $[Ca^{2+}]_i$ changes evoked by kainate in the total population, and in subpopulations 1, 2 and 3

Δ $[Ca^{2+}]_i$ (% of control ± S.E.M.)			
Total population	Subpopulation 1 (< 60%)	Subpopulation 2 (60–90%)	Subpopulation 3 (>90%)
79.9 ± 1.7% (100%)	48.1 ± 3.7% (9.2 %)	79.6 ± 0.9% (73.7 %)	98.4 ± 1.5% (17.1%)

The percentage of hippocampal neurons belonging to each population is indicated in parenthesis

Table 3 Effect of ω-AgaIVA on $[Ca^{2+}]_i$ changes evoked by kainate in the total population, and in subpopulations 1, 2 and 3

Δ $[Ca^{2+}]_i$ (% of control ± SEM)			
Total population	Subpopulation 1 (<60%)	Subpopulation 2 (60–90%)	Subpopulation 3 (>90%)
82.8 ± 2.2% (100%)	51.7 ± 2.9% (16.7%)	72.3 ± 1.5% (66.7%)	95.8 ± 1.8% (16.6%)

The percentage of hippocampal neurons belonging to each population is indicated in parenthesis

Discussion

We have previously shown that VSCCs, particularly L- and N-type Ca^{2+} channels, have a large contribution to the $[Ca^{2+}]_i$ changes evoked by kainate, measured in populations of cultured hippocampal neurons [13]. We also demonstrated that the alterations in the $[Ca^{2+}]_i$ evoked by kainate or AMPA, in individual neurons, present a high cell-to-cell variability, and, in addition, hippocampal neurons have differential sensitivities to LY 303070 and cyclothiazide, which are, respectively, an antagonist and an allosteric modulator of AMPA receptors [14]. Those observations suggest that there is a differential expression and localization of AMPA receptors in hippocampal neurons. Since VSCCs are important mediators of glutamatergic neurotransmission and contribute to $[Ca^{2+}]_i$ changes upon glutamate receptor activation, in the present work we investigated whether different types of VSCCs are differentially coupled to the activation of AMPA and kainate receptors, in single hippocampal neurons.

As previously demonstrated, there was a high cell-to-cell variability in the $[Ca^{2+}]_i$ changes evoked by kainate, again indicating that AMPA and kainate receptors are differentially distributed in hippocampal neurons. The $[Ca^{2+}]_i$ changes were due to AMPA/kainate receptor activation, since NBQX, the AMPA receptor antagonist, abolished the $[Ca^{2+}]_i$ responses, which were not affected by the presence of D-AP5, the NMDA receptor antagonist. Endogenous glutamate, released upon AMPA/kainate receptor activation, could activate NMDA receptors, which might in turn further contribute to the Ca^{2+} signal. However, the blockade of NMDA receptors did not inhibit the $[Ca^{2+}]_i$ changes, indicating that NMDA receptors do not contribute to the $[Ca^{2+}]_i$ changes in these conditions, as was shown previously with $^{45}Ca^{2+}$ uptake experiments and AMPA receptor stimulation in hippocampal neurons [17].

L-type Ca^{2+} channels are particularly abundant in cell bodies and dendrites [3, 18], and several reports have shown that L-type Ca^{2+} channels are activated upon cell stimulation with kainate [19–21]. The present results confirm that these Ca^{2+} channels have an important role in coupling AMPA/kainate receptor activation with the Ca^{2+} signal. Indeed, considering the entire population studied, the inhibitory effect caused by nitrendipine (39.5%) was much higher than that caused either by ω-CgTx GVIA (20.1%) or ω-Aga IVA (17.2%). In addition, the $[Ca^{2+}]_i$ responses evoked by kainate, in the presence of nitrendipine, decreased below 60% of the control in about 40% of neurons. In the presence of either ω-CgTx GVIA or ω-Aga IVA, the $[Ca^{2+}]_i$ responses decreased below 60% of the control in only 9 or 17% of neurons, respectively, much lower than 40% observed for nitrendipine. These facts clearly demonstrate that L-type Ca^{2+} channels have a predominant role in the $[Ca^{2+}]_i$ transients evoked by kainate, in the cell bodies of hippocampal neurons.

It has been demonstrated that N- and P/Q-type Ca^{2+} channels are predominantly located in dendrites and nerve terminals, where they are closely associated with the release machinery [9, 22, 23]. The blockade of these channels has been shown to inhibit the release of several neurotransmitters [24–27] and inhibits glutamatergic synaptic transmission in the hippocampus [23, 28]. We have also previously shown that these channels have a major contribution to the $[Ca^{2+}]_i$ signal in isolated hippocampal nerve terminals [29]. However, in these cultures, N- and P/Q-type Ca^{2+} channels also contributed to the $[Ca^{2+}]_i$ increase in cell bodies, in several neurons, since the $[Ca^{2+}]_i$ responses were inhibited either by ω-CgTx GVIA or ω-Aga IVA in those neurons. These observations clearly indicate that these channels are located in cell bodies and dendrites, at least in some hippocampal neurons. Indeed, it has been demonstrated that both N- and P/Q-type Ca^{2+}

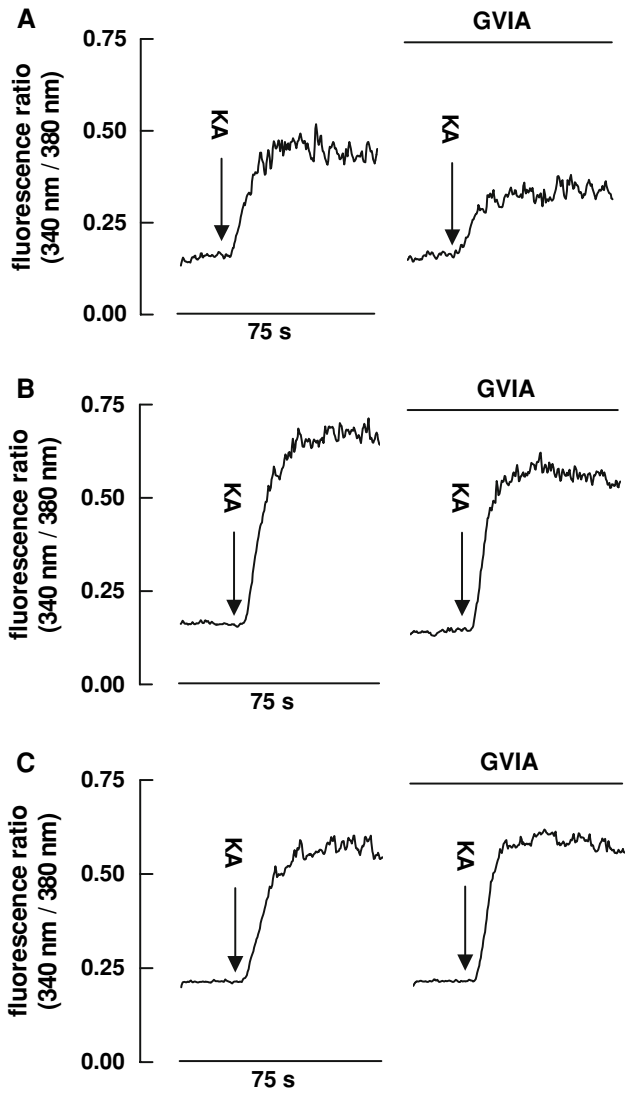

Fig. 3 Effect of ω-GVIA (N-type Ca^{2+} channel blocker) on the $[Ca^{2+}]_i$ changes evoked by kainate (100 μM) in single hippocampal neurons. The results are expressed as the ratio of fluorescence intensity with excitation at 340 and 380 nm (emission at 510 nm) versus time. Each experiment consists of two independent recordings (75 s each), and the first recording was considered an internal control for each cell. The $[Ca^{2+}]_i$ changes were considered as the differences between the peak, after stimulus, and the basal value of the 340 nm/380 nm fluorescence ratio. The $[Ca^{2+}]_i$ changes were analyzed in cell bodies. ω-GVIA (500 nM) was present 5 min before the second stimulus, until the end of each experiment. Since the inhibitory effect of ω-GVIA was heterogeneous, the cells were separated in three subpopulations. The recordings above are representative of each cell population with $[Ca^{2+}]_i$ responses below 60% of the control (**A**), between 60% and 90% of the control (**B**), and above 90% of the control (**C**). A total of 76 neurons, from three independent cell cultures, were analyzed, and the quantification of the data is shown in Table 2

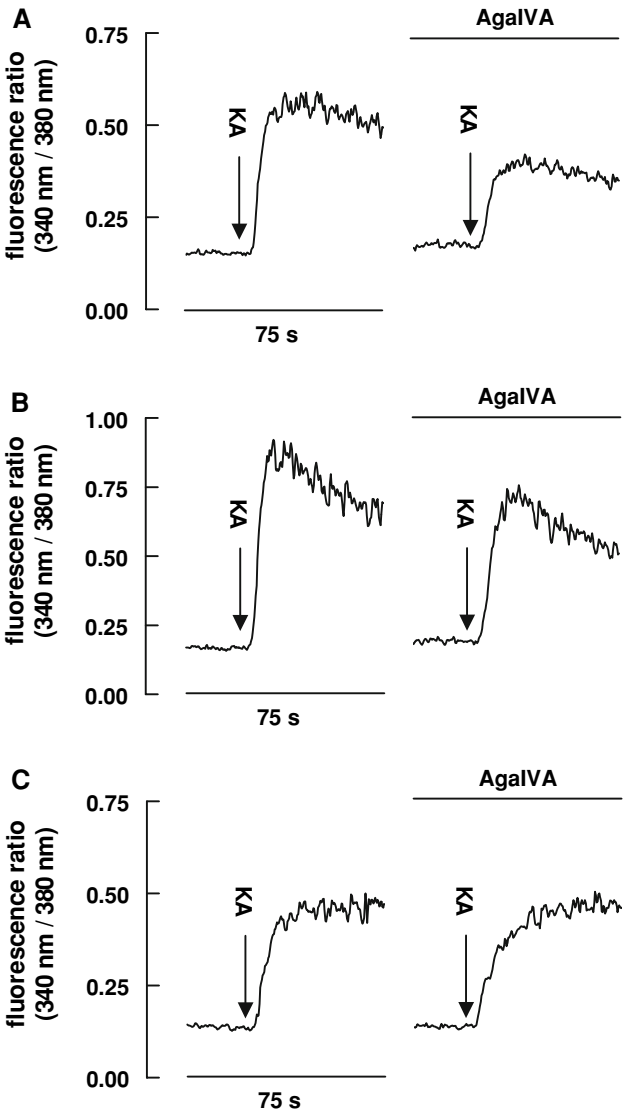

Fig. 4 Effect of ω-AgaIVA (P/Q-type Ca^{2+} channel blocker) on the $[Ca^{2+}]_i$ changes evoked by kainate (100 μM) in single hippocampal neurons. The results are expressed as the ratio of fluorescence intensity with excitation at 340 and 380 nm (emission at 510 nm) versus time. Each experiment consists of two independent recordings (75 s each), and the first recording was considered an internal control for each cell. The $[Ca^{2+}]_i$ changes were considered as the differences between the peak, after stimulus, and the basal value of the 340 nm/380 nm fluorescence ratio. The $[Ca^{2+}]_i$ changes were analyzed in cell bodies. ω-AgaIVA (100 nM) was present 5 min before the second stimulus, until the end of each experiment. Since the inhibitory effect of ω-AgaIVA was heterogeneous, the cells were separated in three subpopulations. The recordings above are representative of each cell population, with $[Ca^{2+}]_i$ responses below 60% of the control (**A**), between 60% and 90% of the control (**B**), and above 90% of the control (**C**). A total of 48 neurons, from three independent cell cultures, were analyzed, and the quantification of the data is shown in Table 3

channels are located in cell bodies and dendrites of several types of neurons, including hippocampal neurons, in addition to their location in nerve terminals [6, 8, 18, 30–32]. Also, these channels contribute to the stimulus-induced postsynaptic Ca^{2+} fluxes in hippocampal neurons [33], and N-type Ca^{2+} channels are primarily involved in

the $[Ca^{2+}]_i$ increase evoked by high potassium in the pyramidal neurons of the dorsal cochlear nucleus [34]. This group of findings indicates that, at least in some cells, N- and P/Q-type Ca^{2+} channels have an important role in glutamatergic synaptic transmission, not only because they regulate transmitter release from nerve terminals, as previously demonstrated, but also because they mediate Ca^{2+} entry in postsynaptic neurons upon the activation of ionotropic glutamate receptors.

In the present work, we showed that the contribution of different types of VSCCs to the $[Ca^{2+}]_i$ signal evoked by kainate is very heterogeneous. In a few cells, the $[Ca^{2+}]_i$ changes were inhibited by about 60% either by nitrendipine, ω-CgTx GVIA or ω-Aga IVA, and in others these blockers were without effect. In some of those cells, either L-, N-, or P/Q-type Ca^{2+} channels are clearly important in coupling glutamate receptor activation with Ca^{2+} signal, probably because these Ca^{2+} channels are particularly abundant in cell bodies and dendrites of those cells or also because both AMPA/kainate receptors and one certain type of Ca^{2+} channels are located in near proximity. In other cells, N- and P/Q-type Ca^{2+} channels did not contribute to the $[Ca^{2+}]_i$ changes or had a low contribution, likely because these Ca^{2+} channels are not located or their expression is rather low in the cell bodies and dendrites of those neurons.

In summary, our results show that the activation of L-type Ca^{2+} channels is coupled to non-NMDA glutamate receptor activation in the cell bodies of hippocampal neurons, and these channels are also a major route for Ca^{2+} entry, although its contribution varies from cell to cell. Regarding N- or P/Q-type Ca^{2+} channels, the results indicate that they are not present in the cell bodies or dendrites of all hippocampal neurons. In some cells, they have an important role in coupling AMPA/kainate receptor activation to Ca^{2+} increase, but in others these Ca^{2+} channels do not contribute at all to Ca^{2+} entry into the cell bodies. The fact that different types of Ca^{2+} channels are differentially coupled to the activation of non-NMDA receptors will certainly have consequences in terms of information processing in neurons and will contribute to variations in cell responses in hipoccampal and brain circuitries.

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